PHYSIOLOGY, HAEMODYNAMICS AND BOLD SIGNALS

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During the past few years NMR methods have greatly contributed to the advancement of cognitive neurosciences and have gained an unprecedented role in modern neurosciences. The avenue was paved by early interest in applying ^{31}P NMR spectroscopy for brain energetics studies [1], followed by introduction of ^{1}H NMR spectroscopy [2, 3] for non-invasive detection of several cerebral metabolites. The great value of ^{13}C NMR for non-invasive neurochemistry was demonstrated in the late 80's [4]. Multinuclear NMR techniques have provided unique information from brain metabolism *in vivo* during baseline and activated states, such as from energy state, intracellular pH (pH_i), glucose metabolism and neurotransmitter glutamate compartmentalisation [5].

By far, MRI techniques play the greatest impact in modern neuroimaging both in clinical and research settings. There are a number of MRI techniques available providing data from cerebral haemodynamics in a truly non-invasive fashion, yet blood oxygenation level dependent (BOLD) MRI is the most commonly used functional neuroimaging method. BOLD signal, as revealed either by T_2* or T_2 MRI, is a composite robust response of haemodynamics and oxidative metabolism to brain activation. MRI techniques have been developed to directly probe cerebral blood flow (CBF) [6, 7] and cerebral blood volume (CBV) [8], both essential components of the haemodynamic response (HDR), without need for injection of contrast agents, to complement BOLD fMRI for neuroimaging.

In this account physiology of cerebral haemodynamics as well as oxygen and glucose metabolism will be discussed from a view point of user of NMR techniques. The focus will be on CBF and cerebral oxygen metabolism ($CMRO_2$) responses to brain activation, with discussion on adaptations of cerebral metabolism during increased energy demand.

Baseline CBF, glucose metabolism and $CMRO_2$

Brain consumes O_2 and glucose at high rates for maintenance of ionic gradients across neural cell plasma membrane. It has been estimated that in human brain close to 85% of ATP consumption is accounted for by post-synaptic ion pumping and action potentials [9]. Substrates for cerebral energy metabolism are supplied by CBF amounting to ~60 ml/min/100g of the brain tissue. Baseline $CMRO_2$, as measured by positron emission tomography (PET), in adult human brain proceeds at 1.5 – 1.7 μmol/min/100 g of tissue [10]. Baseline cerebral metabolic rate of glucose (CMR_{Glu}) by PET is ~ 0.4 μmol/min/100 g of tissue [10]. Using Kety-Schmidt technique, Lund-Madsen et al. showed that the stoicheiometric ratio of $CMRO_2$-to-CMR_{Glu} is, indeed, close to 6 [11]. Thus, this high ratio demonstrates that main body of baseline glucose metabolism is oxidative and only minute portion of glucose (~5%) is metabolised to lactate, consistent with the PET data. Autoradiographic quantification of CBF and CMR_{Glu} has shown large regional variation between gray and white matter as well as between individual brain structures within gray matter [12]. On average CBF is 2- to 3-fold and CMR_{Glu} 3-4-fold greater in gray than in white matter. The difference in these variables has been attributed to the amount of neuronal activity.

Haemodynamics and $CMRO_2$ during brain activation

It is well established with a several techniques that the stoicheiometries between CBF and $CMRO_2$ as well as between $CMRO_2$ and CMR_{Glu} breaks down when neuronal workload increases. In mental processing, stimulated by Wisconsin card sorting test, CBF and CMR_{Glu} increase at global level by 15 and 12%, respectively, however, $CMRO_2$ does not change at all [11]. Arterio-venous difference across the brain increased by 47% during this mental exercise, yet the proportion of glucose appearing in venous blood increased from baseline value of 6% to 8% during brain activation. This indicates that majority of CMR_{Glu} increase is oxidative in nature. PET studies of the somatosensory [10] and visual [13] cortices demonstrated that at focal level CBF and CMR_{Glu} increased by ~50% relative to baseline, whereas only 5-10% elevation in $CMRO_2$ was evident. As a result of these haemodynamic and metabolic adaptations, oxygen saturation in cerebral venous blood increases.

The interrelationship between cerebral oxygen consumption and oxygen delivery can be written as:

$$OER = \frac{CMRO_2}{Y_a \bullet CBF \bullet Hct} \qquad \text{Eq. 1}$$

where OER is oxygen extraction ratio (known also as oxygen extraction fraction = OEF), Y_a the arterial oxygen saturation, and Hct the haematocrit. This relationship is valid under two conditions; firstly, tissue O_2 tension (ptO_2) is assumed to be close to zero and secondly, O_2 transport to the tissue is unidirectional. Both these conditions encounter in the mammalian brain. Considering the observed cerebral CBF and metabolic adaptations above, baseline OER of close to 0.4 must decline during neuronal activation. Indeed, both PET [13] and MRI [14, 15] based methods have shown that OER decreases by 30 to 40% in association to brain activation.

Localised increase in CBF, CBV and decline in OER are ‘physiological substrates’ for non-invasive functional brain imaging techniques. All of these can be quantitatively probed with specific MRI techniques. Why exactly CBF increases beyond the need of $CMRO_2$ during elevated brain workload, is not fully understood, yet physiologic and neurochemical literature dealing with this phenomenon is overwhelming. Primary function of mitochondria is to maintain Gibbs free energy of ATP (ΔG_{ATP}) in cytoplasm within tight limits. For instance, in myocardium ΔG_{ATP} is maintained at -60 kJ/mol despite three-fold variation in oxygen consumption [16]. $CMRO_2$ changes only very moderately from baseline to activated state and therefore, one would expect to see also moderate CBF response, if cerebral O_2 consumption and delivery were quantitatively matched. Cerebral resistance arteries are sensitive to numerous ‘messenger’ molecules, such as CO_2, H^+, K^+, NO, adenosine, aracidonic acid metabolites, glutamate [17] and noradrenaline [18]. It is likely that one or several of these substances mediate the vasodilatory signal from ‘neural cells’ to the vasculature.

Microelectrode measurements of ptO_2 have revealed interesting spatial variation in the brain parenchyma [19, 20]. In the cat visual pathway simultaneous recording of ptO_2 and single cell activity shows that ptO_2 decreases in the sites with increased neuronal spike rate followed by an overshoot with or without a subsequent undershoot in ptO_2 [19]. Microelectrode recordings have shown that the type of visual stimulus determines the shape of ptO_2 response curve. For instance, in a single neuron variation of orientation angle of the light stimulus either elicits firing accompanied with ptO_2 changes described above or does not activate the cell, but leads to elevation of ptO_2 [20]. These results

are interpreted to indicate that (i) neuronal firing declines ptO_2, because CBF response is delayed, and (ii) in some other cases, CBF response 'exceeds' the territory of activated area causing 'luxury' oxygenation of the tissue outside activated area. These findings are very important in the light of physiological mechanisms of CBF response and interpretation of oxygenation changes in neurophysiology equivalents showing that (a) CBF increase is delayed relative to neuronal firing, (b) there is an early deoxygenation of tissue and consequently, also of blood, and (c) ptO_2 can increase without preceding neuronal firing and therefore, CBF can increase in areas with baseline brain activity. One may conclude from the microelectrode work that tissue deoxygenation would be a good marker for imaging of neuronal activity, instead to the delayed 'over-oxygenation', which is associated with CBF response with little, if any, neuronal stimulation.

Optical imaging work using haemoglobin as an endogenous source of signal have revealed that almost immediately following the onset of stimulation, an increase in [Hb] occurs in rat somato-sensory [21] and barrel cortex [22]. Concurrent monitoring of CBF by Laser-Doppler has shown that increase in CBF begins a second or so after first signs of local increase in [Hb]. Following CBF response reciprocal increase in [Hb-O_2] and decrease in [Hb] are evident together with overall elevation of CBV [22]. Interestingly, deoxygenation of both ptO_2 and haemoglobin, as determined by two different methods, appear to coincide preceding the CBF response. This means that $CMRO_2$ must increase almost instantaneously to neuronal firing, consistent with the thermodynamic role of mitochondrial bioenergetics illustrated above. The findings cited above also suggest that $CMRO_2$ response, as reflected by deoxygenation of ptO_2, localize neuronal activity (indicated by single cell firing) much more accurately than any other endogenous component of metabolism or HDR, such as ptO_2 'over-oxygenation' or accumulation of [Hb-O_2]. Importantly, these data also imply that CBF (i.e. perfusion) response is not better localized to the activation site than build up of [Hb-O_2], contrasting a commonly accepted concept in fMRI literature.

The physiology of CBF and $CMRO_2$ during neuronal activation raises a fundamental question: why is there a breakdown of the mismatch between CBF and $CMRO_2$ during increased workload in the brain. This question remains unanswered for time being. Several hypotheses have been put forward to address the mismatch (see [23, 24]), all of these are supported by experimental evidence. In this context, two views are considered. Firstly, the 'energy hypothesis' and 'neurotransmission hypothesis'. The former claims that O_2 requirement of mitochondrial energy metabolism *directly* results in excessive CBF response. One proposed underpinning reason for mismatch of CBF and $CMRO_2$ may be limited oxygen diffusion in the tissue placing high CBF requirements to satisfy need by the oxidative metabolism. There are several potential messengers from the energy metabolism to the resistance arteries, such as CO_2 and H^+. The latter hypothesis stipulates that neurotransmission as such, in the form of neurotransmitters and/or neuromodulators, leads to opening of resistance arteries. In this scenario, cerebral O_2 needs will become (over)satisfied as a result of vasodilatation caused by factors of secondary importance for metabolism. There are a number of neurotransmitters, such as glutamate, adenosine, NO and noradrenaline, with documented effects on vascular resistance.

BOLD signal

Light absorption properties of blood are strongly affected by oxygen saturation, as we all recognise. It became evident early on that also transverse relaxation rate (R_2) in blood is both haemotocrit and oxygen saturation dependent [25, 26]. Paramagnetic Hb inside intact erythrocytes generates field

gradients that affect transverse relaxation of water diffusing in the vicinity of erythrocytes and/or exchanging between plasma and erythrocyte cytoplasm [27]. In deoxygenated blood R_2 (and inevitably also R_2*) is more efficient than in oxygenated blood. Thus, it is conceivable that T_2*-weighted signal from brain parenchyma increases in the activated brain areas as a result of focal decline in OER and concomitant increase in oxygen saturation of postcapillary blood. This BOLD fMRI signal characteristic, referred to as the 'positive BOLD', is the most commonly used contrast in modern functional brain imaging studies.

The BOLD signal has several well documented time-dependent characteristics. Firstly, there is a delay up to several seconds after stimulus onset prior to start of the positive signal deflection. Secondly, several recent studies performed at fields ranging from 1.5T [28], 4 T [29] 7 T[30] to 9.4 T [31] have revealed a negative signal deflection that precedes the positive BOLD response. Interestingly, microelectrode studies have indicated that deoxygenation in ptO_2 very much coincides the initial dip by MRI. Finally, following the positive BOLD signal a negative long-lasting deflection is evident. This is the so-called BOLD post-stimulus undershoot [32]. The post-stimulus undershoot has typically similar or even longer duration than the positive BOLD.

Interplay of haemodynamic and metabolic factors on BOLD signal characteristics has been extensively studied and several 'models' [33-35] have been created to explain their mutual interactions on T_2(*)-weighted fMRI signal. It is commonly accepted that the positive BOLD results from prolongation of T_2(*) in post-capillary blood caused by decline in [Hb] due to decreased OER together with increase in local CBV. The effect of CBV is understandable, because at low and intermediate field strengths blood T_2(*) is longer than that of tissue.

In contrast, physiology underlying both initial dip and post-stimulus undershoot is subject to debate. It has to be appreciated that the initial dip is very difficult to be reliably detected by fMRI and special care is needed both for acquisition of high signal-to-noise-ratio data [36], physiological noise reduction and image processing [30]. Both optical imaging and microelectrode studies have shown almost instantaneous deoxygenation of both haemoglobin and tissue preceding the excessive CBF response. These observations underscore temporal mismatch between $CMRO_2$ and CBF responses very soon after neural activation. The current consensus is that the initial dip reflects local build-up of [Hb] before CBF increase. The echo time dependency of initial dip signal size is consistent with contribution of [Hb] accumulation to the early negative fMRI signal deflection [37].

The BOLD post-stimulus undershoot is very commonly detected by standard T_2*-weighted fMRI. In their papers published in mid 90s, Frahm and co-workers termed the undershoot signal after positive BOLD as 'a negative uncoupling' [32]. The rationale behind this term lays in observations that during BOLD post-stimulus undershoot in the human visual cortex CBF, as determined by MRI, proceeded at baseline level [32]. Several other fMRI studies have confirmed that CBF returns to baseline much before termination of post-stimulus undershoot [38, 39]. Lu et al. quantified also CBV with two second temporal resolution in the visual cortex [39]. They reported that also CBV returns to baseline soon after positive BOLD response, following similar kinetics to CBF. Lu et al. estimated $CMRO_2$ from the multimodal fMRI data during post-stimulus undershoot [39]. The data showed persistent high $CMRO_2$ during BOLD undershoot with baseline CBF. These observations argue for metabolic cause for the undershoot, i.e. build up of [Hb]. In this regard, an interesting observation is that following mental activation, global metabolism of O_2 and glucose remains persistently perturbed [11].

A haemodynamic background for the BOLD post-stimulus undershoot has been put forward by Mandeville et al. [40] and Buxton and Frank [23]. Working with anaesthetised rats, Mandeville and co-workers observed that CBV, as quantified by blood-pool contrast agent based MRI, returns to baseline much later than BOLD undershoot [40]. The balloon model by Buxton and Frank claims that venous blood volume stays elevated some time after stimulus cessation. Mandeville et al. modified the model to incorporate delayed (venous) compliance into the model [40]. Hoge et al. found that in the human visual cortex positive BOLD signal and post-stimulus undershoot were associated with similar transients in CBF, as determined with MRI techniques [41]. They concluded that CBV was returning slowly to baseline during post-stimulus undershoot consistent with the data by Mandeville. Recent high spatial resolution BOLD fMRI and CBV imaging have indicated that CBV may or may not be elevated in cortical layers. Yacoub et al. found that in the cat visual field 18 BOLD undershoot was evident in both parenchyma and brain surface vessel area, yet CBV stayed elevated only in parenchyma [42]. Thus, physiological mechanisms for the BOLD post-stimulus undershoot may vary within brain cortex.

BOLD and baseline flow

Degree of mismatch between $CMRO_2$ and CBF is a key factor determining the size of BOLD signal locally. Baseline CBF may influence the BOLD response and in such case, it would be important to be known, because several brain activation paradigms, such as Wilcoxon card sorting test [11], cognitive and motor task [43], lead to increase in global CBF. The relationship between baseline (global) CBF and (local) BOLD signal characteristics has been experimentally approached during hypo- and hypercapnia. PET techniques were used to quantify CBF both at baseline and during visual activation [44, 45]. Kemna and co-workers found that in mild hypocapnia CBF response was slightly smaller than in normocapnia, whereas in hypercapnia, stimulation induced flow increase was nearly absent [45]. These data are interpreted in the context of the so-called constant relative model. Using PET imaging, Friston et al. reported that CBF responses to stimulation are additive to global CBF [46], thus supporting the so-called additive model.

One can find support for these two models in fMRI literature. Hoge and co-workers found that both BOLD and perfusion responses during hypercapnia were additive in the visual cortex [47]. These results, together with the paper by Corfield et al. [48], argue for the additive effect of CBF on BOLD response. Cohen et al. studied BOLD characteristics in the visual cortex at 7 T [49]. They demonstrated that size of positive BOLD signal became greater in hypocapnia and smaller in hypercapnia, than that determined in normocapnia. Further, BOLD signal width was smaller and wider during former and latter challenge, respectively. This study supports the constant relative model of the HDR. Clearly, more controlled data is needed to settle this issue. Nevertheless, these papers underscore the impact of baseline CBF on BOLD fMRI data interpretation in terms of brain activity.

BOLD and vessel type contributions

Changes in blood oxygenation due to the HDR are greatest in the post-capillary and draining veins. Therefore it is not surprising that venous vasculature strongly contributes to the BOLD signal. This is particularly evident at low and intermediate field strength, where both gradient echo and spin echo fMRI signal gains strong contributions from macrovasculature [50-52]. One can reduce the contribution

by macrovasulature by using flow dephasing gradients [53], high field system [51] with optimal echo times [54]. A further effect high field (B_0 >3T) exerts on BOLD is a decline in intravascular contribution [55].

Mechanisms linking HDR and neuronal activation

MRI techniques provide a good handle to cerebral haemodynamics and oxygenation, however, these are only indirectly linked to brain activity. It is not surprising if one finds split views in fMRI literature regarding mechanisms of coupling between CBF, $CMRO_2$ and brain activation. Substantial number of papers supports [23, 33, 48] the energy hypothesis, i.e. control of the HDR by needs of mitochondrial energy metabolism. Similarly, recent multi-modal fMRI studies argue for mechanisms of CBF regulation that are independent of cerebral oxygen metabolism [39]. Recent fMRI experiments performed in subjects exposed to mild hypoxia during brain activation have indicated heterogeneity in BOLD signal size within the activated brain area [56]. These observations point to heterogeneity in OER within the activated cortex, suggesting that inherent heterogeneity in brain oxygen delivery and metabolism shown by microelectrode studies [19, 20] complicate interpretation of BOLD results in terms of underlying (neuro)physiology.

Glucose metabolism during brain activation by NMR spectroscopy

Both ^{31}P and ^{1}H NMR spectroscopy have been used to explore cerebral energy metabolites and lactate during brain activation. A decline in PCr/P_i ratio was detected by ^{31}P NMR during exhaustive visual activation [57]. ^{1}H NMR spectroscopy provides access to lactate by revealing a $-CH_3$ at 1.33 ppm. This doublet resonance is found in the chemical shift region with few, if any, other cerebral metabolites, yet residual signal from extracerebral lipids may encode to this spectral region. As indicated above, brain activation studies have shown that at global level CMR_{Glu} increases and majority of this increase is oxidative [11]. PET data, however, show that local CMR_{Glu} go up much more than local $CMRO_2$ indicating aerobic lactate accumulation. Microdialysis work has revealed lactate extrusion to the interstitial space during brain stimulation in rats [58], a finding agreeing with the PET scans. ^{1}H NMR studies have shown all possible directions for brain lactate change during activation. Some studies have demonstrated no change [59], some an increase [57, 60] and finally, some a decrease [59] in NMR detectable lactate. Interestingly, visual activation during mild hypoxia, when evoked brain responses are retained at euoxic levels, does not result in accumulation of ^{1}H NMR detectable lactate [56, 61]. These data, as supported by ^{1}H NMR spectroscopy papers above, fit to the idea that glucose metabolism during increased brain activity is oxiditative.

Recent NMR spectroscopy studies exploiting ^{13}C-labelled glucose have provided interesting neurochemical information from cerebral metabolism during activation. The beauty of using NMR with enriched ^{13}C-glucose is that one can follow glucose metabolism beyond cytoplasmic pathways all the way to the tricarboxylic acid cycle (TCA) and beyond [62]. In fact, labeling kinetics of glutamate C4 and C3/C2 has been used to quantify TCA cycle rate, which is ultimately linked to $CMRO_2$. Chen and co-workers [63] exploited indirect detection of ^{13}C-labelled metabolites, including glutamate C4, in the human brain. They estimated that TCA cycle rate increased by ~30% during visual stimulation representing ceiling response for $CMRO_2$. Chhina et al. used direct detection of ^{13}C with NOE enhancement [64]. Following visual stimulation labeling of glutamate C4 increased by 50-60% suggesting that TCA cycle rate increased as much as CMR_{Glu} reported by PET [13]. One has to

realise, however, that PET measures glucose uptake and phosphorylation, not glycolysis. Nevertheless, the fact that no ^{13}C label accumulation was seen in lactate during visual stimulation tend to favour the idea that ΔCMR_{Glu} occurs oxidatively. This observation agrees with the results from human data obtained from arterio-venous difference measurements [11].

Conclusions

Surge of fMRI for cognitive neuroscience has resulted in renaissance of the physiology research of haemodynamics and its relationships with brain activation. NMR techniques are playing a key role in this pursuit and perhaps may finally help to untangle the physiological substrates linking the HDR and neural activity.

Literature cited

1. Ackerman JJH, Grove TH, Wong GG, Gadian DG, Radda GK. *Nature*. 1980; **283**: 167 - 170.
2. Behar KL, den Hollander JA, Stromski ME *et al. Proc Natl Acad Sci USA*. 1983; **80**: 4945-4948.
3. Frahm J, Merboldt KD, Hänicke W. *J Magn Reson*. 1987; **72**: 502-508.
4. Bachelard HS, Badar-Goffer R. *J Neurochem*. 1993; **61**: 412-429.
5. Gruetter R. *Neurochem Int*. 2002; **41**: 143-154.
6. Detre JA, Leigh JS, Williams DS, Koretsky AP. *Magn Reson Med*. 1992; **23**: 37-45.
7. Golay X, Hendrikse J, Lim TC. *Top Magn Reson Imaging*. 2004; **15**: 10-27.
8. Lu H, Golay X, Pekar JJ, Van Zijl PC. *Magn Reson Med*. 2003; **50**: 263-274.
9. Attwell D, Laughlin SB. *J Cereb Blood Flow Metab*. 2001; **21**: 1133-1145.
10. Fox PT, Raichle ME. *Proc Natl Acad Sci USA*. 1986; **83**: 1140 - 1144.
11. Lund Madsen P, Hasselbalch SG, Hagemann LP *et al. J Cereb Blood Flow Metab*. 1995; **15**: 485-491.
12. Sokoloff L. **In**: Siegel G, Arganoff B, Albers RW, Molinoff P (eds.), *Basic Neurochemistry*. Raven Press, New York, 1989; 565-590.
13. Fox PT, Raichle ME, Mintun MA, Dence C. *Science*. 1988; **241**: 462 - 464.
14. Oja JM, Gillen JS, Kauppinen RA, Kraut M, van Zijl PC. *J Cereb Blood Flow Metab*. 1999; **19**: 1289-1295.
15. Golay X, Silvennoinen MJ, Zhou J *et al. Magn Reson Med*. 2001; **46**: 282-291.
16. Kauppinen RA, Hiltunen JK, Hassinen IE. *FEBS Lett*. 1980; **112**: 273 - 276.
17. Zonta M, Angulo MC, Gobbo S *et al. Nat Neurosci*. 2003; **6**: 43-50.
18. Mulligan SJ, MacVigar BA. *Nature*. 2004; **431**: 195-199.
19. Thompson JK, Peterson MR, Freeman RD. *Science*. 2003; **299**: 1070-1072.
20. Thompson JK, Peterson MR, Freeman RD. *Nat Neurosci*. 2004; **7**: 919-92-.
21. Malonek D, Grinvald A. *Science*. 1996; **272**: 551-554.
22. Jones M, Berwick J, Johnston D, Mayhew J. *Neuroimage*. 2001; **13**: 1002-1015.
23. Buxton RB, Frank LR. *J Cereb Blood Flow Metab*. 1997; **17**: 64-72.
24. Attwell D, Iadecola C. *Trends Neurosci*. 2002; **25**: 621-625.
25. Brindle KM, Brown FF, Cambell ID, Grathwohl C, Kuchel PW. *Biochem J*. 1979; **180**: 37-44.
26. Thulborn KR, Waterton JC, Matthews PM, Radda GK. *Biochim Biophys Acta*. 1982; **714**: 265-270.
27. Jensen JH, Chandra R. *Magn Reson Med*. 2000; **44**: 144-156.
28. Yacoub E, Hu X. *Magn Reson Med*. 1999; **41**: 1088-92.
29. Yacoub E, Hu X. *Magn Reson Med*. 2001; **45**: 184-190.
30. Yacoub E, Shmuel A, Pfeuffer J *et al. NMR Biomed*. 2001; **14**: 408-412.
31. Kim DS, Duong TQ, Kim SG. *Nat Neurosci*. 2000; **3**: 164-169.
32. Frahm J, Kruger G, Merboldt KD, Kleinschmidt A. *Magn Reson Med*. 1996; **35**: 143-148.
33. Hoge RD, Atkinson J, Gill B, Crelier GR, Marrett S, Pike GB. *Proc Natl Acad Sci U S A*. 1999; **96**: 9403-9408.
34. Zheng Y, Martindale J, Johnston D, Jones M, Berwick J, Mayhew J. *Neuroimage*. 2002; **16**: 617-637.
35. Buxton RB, Uludag K, Dubowitz DJ, Liu TT. *Neuroimage*. 2004; **23**: S220-S233.
36. Ernst T, Hennig J. *Magn Reson Med*. 1994; **32**: 146-149.

37. Yacoub E, Le TH, Ugurbil K, Hu X. *Magn Reson Med*. 1999; **41**: 436-441.
38. Obata T, Liu TT, Miller KL *et al. Neuroimage*. 2004; **21**: 144-153.
39. Lu H, Golay X, Pekar JJ, Van Zijl PC. *J Cereb Blood Flow Metab*. 2004; **24**: 764-770.
40. Mandeville JB, Marota JJ, Kosofsky BE *et al. Magn Reson Med*. 1998; **39**: 615-624.
41. Hoge RD, Atkinson J, Gill B, Crelier GR, Marrett S, Pike GB. *Neuroimage*. 1999; **9**: 573-585.
42. Yacoub E, Ugurbil K, Harel N. *J Cereb Blood Flow Metab*. 2005; **in press**.
43. Moody M, Panerai RB, Eames PJ, Potter JF. *Am J Physiol*. 2005; **288**: R1581-R1588.
44. Shimosegawa E, Kanno I, Hatazawa J *et al. J Cereb Blood Flow Metab*. 1995; **15**: 111-114.
45. Kemna LJ, Posse S, Tellmann L, Schmitz T, Herzog H. *J Cereb Blood Flow Metab*. 2001; **21**: 664-670.
46. Friston KJ, Frith CD, Liddle PF, Dolan RJ, Lammertsma AA, Frackowiak RS. *J Cereb Blood Flow Metab*. 1990; **10**: 458-466.
47. Hoge RD, Atkinson J, Gill B, Crelier GR, Marrett S, Pike GB. *Magn Reson Med*. 1999; **42**: 849-863.
48. Corfield DR, Murphy K, Josephs O, Adams L, Turner R. *Neuroimage*. 2001; **13**: 1207-1211.
49. Cohen ER, Ugurbil K, Kim SG. *J Cereb Blood Flow Metab*. 2002; **22**: 1042-1053.
50. Haacke EM, Hopkins A, Lai S *et al. NMR Biomed*. 1994; **7**: 54-62.
51. Gati JS, Menon RS, Ugurbil K, Rutt BK. *Magn Reson Med*. 1997; **38**: 296-302.
52. Oja JME, Gillen J, Kauppinen RA, Kraut M, van Zijl PCM. *Magn Reson Med*. 1999; **42**: 617-626.
53. Song AW, Wong EC, Tan SG, Hyde JS. *Magn Reson Med*. 1996; **35**: 155-158.
54. Silvennoinen MJ, Clingman CS, Golay X, Kauppinen RA, van Zijl PCM. *Magn Reson Med*. 2003; **49**: 47-60.
55. Lu H, van Zijl PCM. *Proc Intl Soc Magn Reson Med*. 2004; 275.
56. Tuunanen PI, Murray IJ, Parry NRA, Kauppinen RA. *J Cereb Blood Flow Metab*. 2005; **in press**.
57. Sappey-Marinier D, Calabress G, Fein G, Hugg JW, Biggins C, Weiner MW. *J Cereb Blood Flow Metab*. 1992; **12**: 584 - 592.
58. Kuhr WG, Korf J. *J Cereb Blood Flow Metab*. 1988; **8**: 130-137.
59. Merboldt KD, Bruhn H, Hänicke W, Michaelis T, Frahm J. *Magn Reson Med*. 1992; **25**: 187 - 194.
60. Prichard J, Rothman D, Novotny E *et al. Proc Natl Acad Sci USA*. 1991; **88**: 5829 - 5831.
61. Kauppinen RA, Eleff SM, Ulatowski JA, Kraut M, Soher B, van Zijl PCM. *Eur J Neurosci*. 1997; **9**: 654-661.
62. Gruetter R, Adriany G, Choi IY, Henry PG, Lei H, Oz G. *NMR Biomed*. 2003; **16**: 313-338.
63. Chen W, Zhu XH, Gruetter R, Seaquist ER, Adriany G, Ugurbil K. *Magn Reson Med*. 2001; **45**: 349-355.
64. Chhina N, Kuestermann E, Halliday J *et al. J Neurosci Res*. 2001; **66**: 737-746.